

PENDING CLAIMS

71. A method of detecting a nucleic acid, comprising:

a) providing:

- i) a cleavage means;
- ii) a target nucleic acid, said target nucleic acid comprising a first region and a second region, said second region downstream of said first region;
- iii) a first oligonucleotide comprising a 3' portion and a 5' portion;
- iv) a second oligonucleotide; and
- v) a third oligonucleotide;

b) mixing said cleavage means, said target nucleic acid, said first oligonucleotide, and said second oligonucleotide under conditions such that at least said 3' portion of said first oligonucleotide is hybridized to said first region of said target nucleic acid and wherein at least a portion of said second oligonucleotide is hybridized to said second region of said target nucleic acid to form a first complex, and wherein said cleavage of said first complex by said cleavage means liberates said 5' portion of said first oligonucleotide as a first cleavage product;

c) reacting said first cleavage product with said third oligonucleotide and said cleavage means such that at least a portion of said first cleavage product is hybridized to said third oligonucleotide to form a second complex, wherein cleavage of said second complex generates a second cleavage product; and

d) detecting the cleavage of said second complex.

72. The method of Claim 71, wherein said detecting the cleavage of said second complex comprises detecting said second cleavage product.

73. The method of Claim 71, wherein said conditions comprise isothermal conditions.

74. The method of Claim 71, wherein said detecting the cleavage of said second complex comprises detection of fluorescence.

75. The method of Claim 71, wherein said detecting the cleavage of said second complex comprises detection of mass.

76. The method of Claim 71, wherein said second complex comprises a fluorophore having quenched emission, and wherein said detecting the cleavage of said second complex comprises detection of an increase in fluorescence intensity.

77. The method of Claim 71, wherein said detecting the cleavage of said second complex comprises detection selected from the group consisting of detection of radioactivity, luminescence, dye intercalation, fluorescence polarization, staining, or color.

78. The method of Claim 71, wherein said first oligonucleotide is attached to a solid support.

79. The method of Claim 71, wherein said second oligonucleotide is attached to a solid support.

80. The method of Claim 71, wherein said third oligonucleotide is attached to a solid support.

81. The method of Claim 71, wherein said cleavage means comprises an enzyme.

82. The method of Claim 81, wherein said enzyme comprises a DNA polymerase.

83. The method of Claim 82, wherein said DNA polymerase comprises a thermostable DNA polymerase.

84. The method of Claim 83, wherein said thermostable DNA polymerase is derived from an organism from genus *Thermus*.

85. The method of Claim 81, wherein said enzyme comprises a 5' nuclease.

86. The method of Claim 81, wherein said enzyme comprises a thermostable 5' nuclease derived from a thermostable DNA polymerase modified to have reduced synthetic activity.

87. The method of Claim 71, wherein said first and said second regions of said target nucleic acid are adjacent to each other.

88. The method of Claim 71, wherein a portion of said second oligonucleotide that is hybridized to said target nucleic acid comprises a 3' terminus.

89. The method of Claim 71, wherein said third oligonucleotide comprises a hairpin structure that comprises a duplex region adjacent to a single-stranded 3' arm.

90. The method of Claim 89, wherein said portion said third oligonucleotide hybridized to said portion of said first cleavage product comprises at least a region of said single-stranded 3' arm of said hairpin structure.

91. The method of Claim 90, wherein said region of said single-stranded 3' arm is adjacent to said duplex region of said hairpin structure.

92. The method of Claim 71, wherein a portion of said first cleavage product that is hybridized to said third oligonucleotide comprises a 3' terminus.

93. The method of Claim 71, wherein said cleavage of said second complex cleaves said third oligonucleotide.

94. The method of Claim 93, wherein said cleavage of said third oligonucleotide is within said duplex region.

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- a) providing:
 - i) a cleavage means;
 - ii) a target nucleic acid, said target nucleic acid comprising a first region and a second region, said second region downstream of said first region;
 - iii) a first oligonucleotide comprising a 3' portion and a 5' portion;
 - iv) a second oligonucleotide; and
 - v) a third oligonucleotide;
 - b) mixing said cleavage means, said target nucleic acid, said first oligonucleotide, and said second oligonucleotide under conditions such that at least said 3' portion of said first oligonucleotide is hybridized to said first region of said target nucleic acid and wherein at least a portion of said second oligonucleotide is hybridized to said second region of said target nucleic acid to form a first complex, and wherein said cleavage of said first complex by said cleavage means liberates said 5' portion of said first oligonucleotide as a first cleavage product;
 - c) reacting said first cleavage product with said third oligonucleotide and said cleavage means such that at least a portion of said first cleavage product is hybridized to said third oligonucleotide to form a second complex, wherein cleavage of said second complex generates a second cleavage product; and
 - d) detecting the cleavage of said second complex.
72. The method of Claim 71, wherein said detecting the cleavage of said second complex comprises detecting said second cleavage product.
73. The method of Claim 71, wherein said conditions comprise isothermal conditions.
74. The method of Claim 71, wherein said detecting the cleavage of said second complex comprises detection of fluorescence.
75. The method of Claim 71, wherein said detecting the cleavage of said second complex comprises detection of mass.

76. The method of Claim 71, wherein said second complex comprises a fluorophore having quenched emission, and wherein said detecting the cleavage of said second complex comprises detection of an increase in fluorescence intensity.

77. The method of Claim 71, wherein said detecting the cleavage of said second complex comprises detection selected from the group consisting of detection of radioactivity, luminescence, dye intercalation, fluorescence polarization, staining, or color.

78. The method of Claim 71, wherein said first oligonucleotide is attached to a solid support.

79. The method of Claim 71, wherein said second oligonucleotide is attached to a solid support.

80. The method of Claim 71, wherein said third oligonucleotide is attached to a solid support.

81. The method of Claim 71, wherein said cleavage means comprises an enzyme.

82. The method of Claim 81, wherein said enzyme comprises a DNA polymerase.

83. The method of Claim 82, wherein said DNA polymerase comprises a thermostable DNA polymerase.

84. The method of Claim 83, wherein said thermostable DNA polymerase is derived from an organism from genus *Thermus*.

85. The method of Claim 81, wherein said enzyme comprises a 5' nuclease.

86. The method of Claim 81, wherein said enzyme comprises a thermostable 5' nuclease derived from a thermostable DNA polymerase modified to have reduced synthetic activity.

87. The method of Claim 71, wherein said first and said second regions of said target nucleic acid are adjacent to each other.

88. The method of Claim 71, wherein a portion of said second oligonucleotide that is hybridized to said target nucleic acid comprises a 3' terminus.

89. The method of Claim 71, wherein said third oligonucleotide comprises a hairpin structure that comprises a duplex region adjacent to a single-stranded 3' arm.

90. The method of Claim 89, wherein said portion said third oligonucleotide hybridized to said portion of said first cleavage product comprises at least a region of said single-stranded 3' arm of said hairpin structure.

91. The method of Claim 90, wherein said region of said single-stranded 3' arm is adjacent to said duplex region of said hairpin structure.

92. The method of Claim 71, wherein a portion of said first cleavage product that is hybridized to said third oligonucleotide comprises a 3' terminus.

93. The method of Claim 71, wherein said cleavage of said second complex cleaves said third oligonucleotide.

94. The method of Claim 93, wherein said cleavage of said third oligonucleotide is within said duplex region.